Preferred Negative Geotactic Orientation in Mobile Cells: *Tetrahymena* Results

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ABSTRACT For the protozoan species *Tetrahymena* a series of airplane experiments are reported, which varied gravity as an active laboratory parameter and tested for corresponding changes in geotaxic orientation of single cells. The airplane achieved alternating periods of low (0.01 *g*) and high (1.8 *g*; $g = 980$ cm/s) gravity by flying repeated Keplerian parabolas. The experimental design was undertaken to clearly distinguish gravity from competing aerodynamic and chemical gradients. In this way, each culture served as its own control, with gravity level alone determining the orientational changes. On average, 6.3% of the *Tetrahymena* oriented vertically in low gravity, while 27% oriented vertically in high-gravity phases. Simplified physical models are explored for describing these cell trajectories as a function of gravity, aerodynamic drag, and lift. The notable effect of gravity on turning behavior is emphasized as the biophysical cause of the observed negative geotaxis in *Tetrahymena*. A fundamental investigation of the biological gravity receptor (if it exists) and improved modeling for vertical migration in important types of ocean plankton motivate the present research.

INTRODUCTION

Gravity has a pronounced effect on biological and cellular functions including cell proliferation (Mergenhagen, 1986), biosynthesis of cell-specific products, consumption of nutrients in the medium (Montgomery et al., 1978), and kinetics of cell differentiation (Gmunder and Gogli, 1988). Despite the long history of interest in the mechanism and effects of gravity on biological function (e.g., Wager, 1911), research on cell mobility and orientation with respect to gravity is still an active field. Recent work (Shvirst et al., 1984) on the protozoa *Tetrahymena* in particular has called into question the traditional mechanisms for geotaxis (or gravitaxis) in upwardly oriented swimming.

We therefore presently consider the effects of gravity sensing on single-cell orientation and quantify directional swimming changes using variable gravity simulations. No air gaps or oxygen gradients were present, so gravity alone acted to determine cell orientation. The research seeks to change actively the gravity level, to monitor photographically the angular orientation of cells, and finally to relate the geometric character of swimming trajectories to the gravitational biology of the organisms themselves.

To investigate gravity effects on orientation of single cells, movements of protozoa *Tetrahymena* were recorded in a vertical cross-section (Fig. 1). *T. pyriformis* was pursued as a principal target organism because its gravitational sensitivity has been characterized previously (Fukui and Asai, 1985; Noever, 1991), and its lack of a photodynamic response effectively eliminates competing light and chemical effects in solution. Its short generation times and thoroughly investigated biology make it what one researcher (Hill, 1972) called a "biochemical star"; its metabolic cycle shares many common features with higher organisms (e.g., vitamin and essential mineral requirements, etc.) As a result, for more than 40 years, *T. pyriformis* has been an organism of choice (Nillson, 1989) for assessing chemosensory and physiological effects. Our own interest in this area is motivated in large part by the importance of vertical migration in the life cycle of ocean plankton (Harrison and Caverhill, 1991; Winet and Jahn, 1972) and by the need for better models governing ocean-atmosphere exchange of greenhouse gases such as carbon dioxide.

MATERIALS AND METHODS

Organisms and culture conditions

The ciliate, *Tetrahymena pyriformis* (American Tissue Type Collection, Rockville, MD) was grown in (autoclaved) 2% proteose-peptone-yeast medium (Starr and Zeikus, 1987). The organisms were cultivated axenically in a temperature-controlled (22°C) clean room (Class III). The protists were grown in 1-l glass containers without additional gassing or mechanical agitation.

Determination of cell orientation and gravity

Cell suspensions were prepared as low-density cultures ($<3.2 \times 10^2$ cells ml^{-1}). Assuming typical *Tetrahymena* cell parameters (20–50% g carbon/g dry weight, 80% water content by weight, and cell specific gravity of 1.05 $g\ ^{cm\text{-}3}$), this cell population corresponds to ~0.001 g of carbon/m^3 (Hill, 1972).

To observe and photograph the swimming motion, cell suspensions were added to a glass vessel whose inside dimensions were 40 mm × 10 mm × 1 mm. Viewing was done in the 40 mm × 10 mm plane. The glass vessel was made from a commercial glass manufactured as low electrically conducting for electrophoresis grade work (Rank Bros., Cambridge, UK). The two-dimensional projections of swimming patterns were recorded by low-magnification (10:1) video (30 frames/s) microscopy under light field. The focal plane of the microscope was adjusted such that the cell outline was traceable throughout the 1 mm depth. No loss of photographic information was observed as cells moved in this vertical (1 mm) depth, and each frame grabbed spanned an approximate viewing size in the plane of 1/40 the entire vessel.

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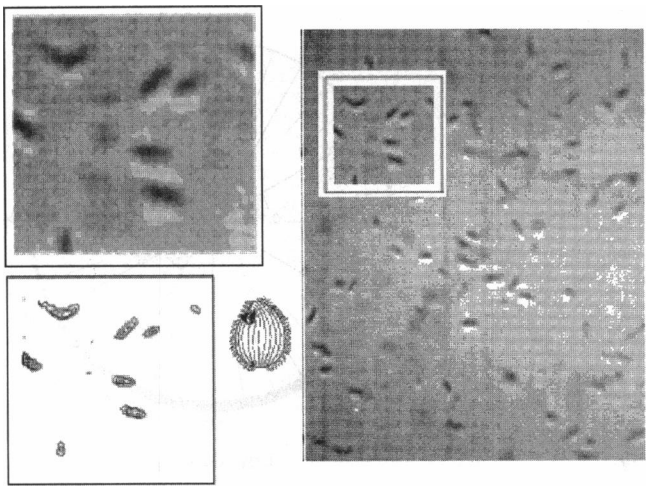

FIGURE 1 Photograph results of single-cell orientation and cell-tracking outlines. The middle right frame shows a quarter view of the microscope area with cells (50 μm). The boxed area is enlarged upper left and contoured for cell outline and identification. In the middle is a schematic cell with ciliary (motion) apparatus.

Transitions in gravity level were marked on the audio portion of the cell video tracks. Accelerometers mounted on the same axis as the viewing recorder gave an instantaneous coupling between applied acceleration and the directional orientation of the major ellipsoidal cell axis. Between successive gravity levels the cell environment remained constant for pH, temperature, osmotic pressure, anaerobic conditions, ultraviolet exposure, surface active components, and viscosity. Because cell age varied over 2 h of variable gravity, comparisons were made between runs separated by less than 20 min. Typical times between successive high-g (1.8 g) and low-g (0.01 g) observation levels was 25–30 s. Changes in linear velocity or in frequency or amplitude of flagellar beating were not considered. No observed changes in body shape or outline were noted in video analysis. Thermal convection was not observed in either non-motile cells of *T. pyriformis* or with 0.1 mm aluminum flakes in water.

Image analysis

The image of cell orientation was recorded on video (Fig. 2). The photographic images were digitized by tracing the ovoid cell surface, then scanned (Albaton 300S scanner, San Mateo, CA) with a spatial resolution of 300 dots per inch. The digital images were further analyzed for geometric parameters of swimming direction using a main image analysis program (Automatix, Cambridge, MA).

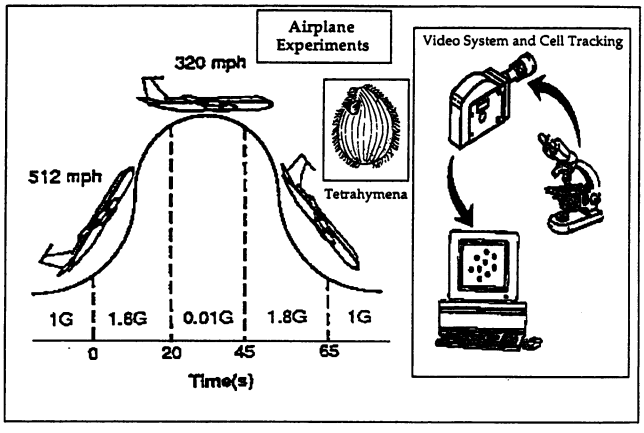

FIGURE 2 Schematic of apparatus and plane trajectory.

The outline and swimming direction of each cell was determined using a chain-coding algorithm and analyzed spatially as a best-fitted centroid. For each cell, the geometry was stored in the form of its ellipsoidal orientation with respect to vertical (arbitrary pixel units) as well as the number of cells in a frame, then calibrated (normalized) to the average value for all cells (average density, 50 cells/frame). As the swimming directions changed with high and low gravity, the geometric measures of cell orientation were plotted as a function of applied accelerations.

Variable gravity protocol

Variable gravity was induced using NASA's KC-135 research aircraft (Fig. 2). It is a modified Boeing 707 Turbo jet. The plane flies a parabolic trajectory and alternatingly achieves 25 s of 10^{-2} g ($\pm 2 \times 10^{-3}$ g) during a pushover phase and 20 s of 1.8–2 g during pullup and pullout phases. Each experimental cycle (low gravity = 0.01 g, unit gravity = 1 g, high gravity = 1.8 g, g = 980 cm s^{-2}) was performed over the course of 2 h during 40 successive phases of high and low gravity. Accelerometer readings of applied accelerations were recorded at 200 Hz in the z axis (vertical). Following takeoff, cabin temperature remained constant at 28°C.

RESULTS

Swimming orientation

Results (Fig. 3) showed that random swimming dominates low-gravity phases while directed upward orientation dominates high gravity phases. This finding is reported as the gravity dependence of angle deviations from vertical cell orientation and average swimming direction. Visual presentation of angular data is shown in Fig. 3, *A* and *B*. Relevant geometric parameters of the two angular distributions of Fig. 3 *A* are compiled in Table 1.

The behavior of live *Tetrahymena* cells contrasts markedly with either dead cells or aluminum flakes. Both the non-motile cells and flakes are seen to gather at the bottom of the glass vessel in unit and high-gravity phases and remain there during short (20 s) low-gravity phases. This observation supports the conclusion that active swimming is essential for a uniform dispersion of organisms, since neither passive diffusion of inorganic material nor any stirring currents that might arise during high-to-low gravity shifts can act to overcome the viscous resistance of the fluid. We also conclude that any small angular changes in the plane trajectory relative to vertical gravity do not have a pronounced effect on the live cells.

A two-dimensional model of swimming direction in gravity fields

Because the focal depth of the viewing apparatus was large, tracking individual cell paths is not possible in three dimensions. A helical trajectory cannot readily be distinguished from planar motion in this set of reported experiments. To model theoretically these effects of gravity in combination with complex swimming patterns, we adapt classical models of motility to a variable gravity field.

Fukui and Asai (1985) have proposed a dynamical description of cell orientation in gravity. The translational motion of the center of gravity of the cell is oriented by a slight (14%) difference in position between the center

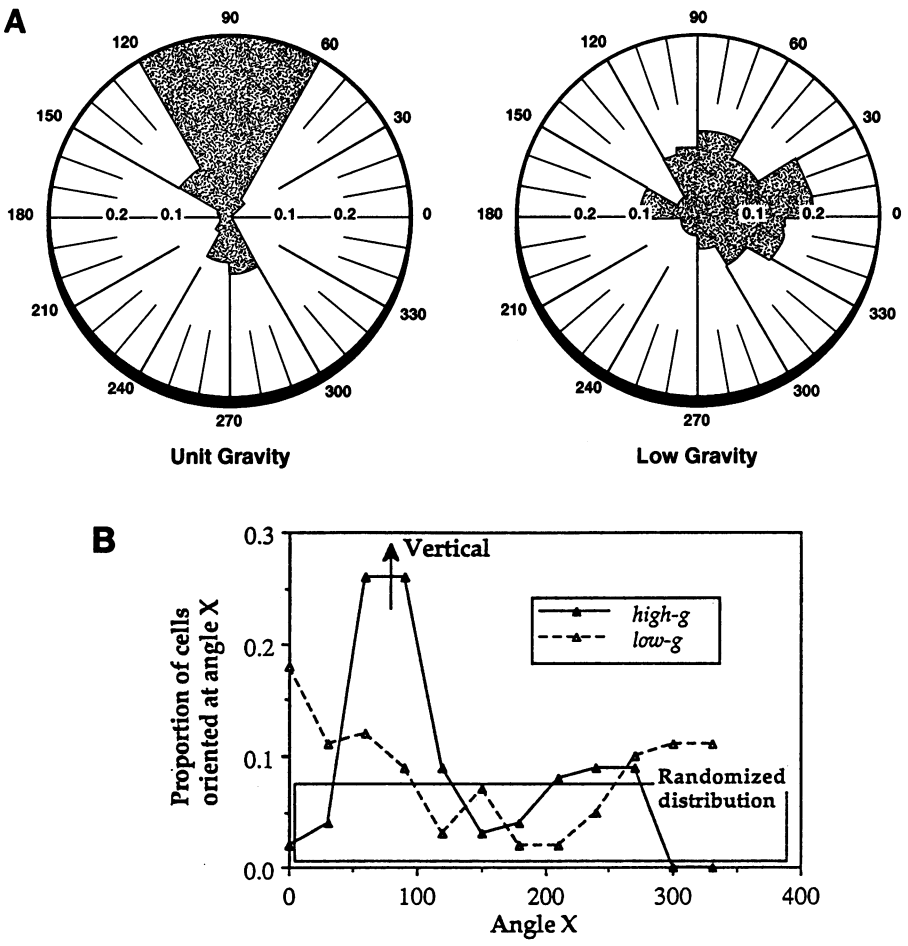

FIGURE 3 Direction of swimming for high and low gravity. *A*) The areas shaded correspond to the number of swimming cells oriented in each 30° interval. *B*) Line graph of the angular distribution for high- and low-gravity orientations. The peak for high gravity is seen to occur at 90° (vertical). The bottom boxed area corresponds to an expected random distribution of 8% of the cells populating each 30° angular bin.

TABLE 1 Statistical comparison of circular angle plots (Fig. 3 A) for various geometric parameters of gravitational asymmetry in organism orientation

g	Gravity level ×980 cm/s	Area	Roundness	Axis ratio	Radius ratio	Length ratio	Minor axis length	Major axis length	Asymmetry (major)	Asymmetry (minor)
High	1.8	1.00	1.00	0.92	1.00	1.00	1.00	1.00	1.00	1.00
Low	0.01	0.63	0.87	1.00	0.78	0.87	0.81	0.74	−0.35	0.38

The shaded regions in the figure, which correspond to an angular interval for the number of cells populating that angle bin, were analyzed for their space-filling properties and directional skewness.

of gravity and the center of buoyancy. The result is rotational motion of the organism's body about its center of gravity. In two dimensions, a cell traces a swimming trajectory that depends on gravity according to the simple force balance

$$M\frac{d^2x}{dt^2} + R\frac{dx}{dt} = P\cos\vartheta \tag{1}$$

in the horizontal coordinate,

$$M\frac{d^2y}{dt^2} + R\frac{dx}{dt} = P\sin\vartheta - mg \tag{2}$$

in the vertical coordinate, and the angular momentum balance for the angle of attack as

$$I\frac{d^2\vartheta}{dt^2} + n\frac{d\vartheta}{dt} = T\cos\vartheta. \tag{3}$$

These equations physically describe the ballistic flight of a self-propelled object with organism mass M, effective mass or density differential m, translational friction coefficient R, rotational friction coefficient n, angle of attack between horizontal and vertical swimming ϑ, swimming or propulsive force P, and moment of inertia I. The following *Tetrahymena* parameters (Hill, 1972) were used. $R = 6\pi\eta a$, where a = the organism's mean radius (0.005 cm), $P = 7.07 \times 10^{-5}$ dyn; $m = 0.05$ g cm^{-3} and $T/n = 0.13$ s^{-1}).

$$x = x_o + \frac{nP}{TR}\cos\vartheta \tag{4}$$

$$y = y_o - \frac{nP}{TR}\ln\cos\vartheta - \frac{nmg}{TR} \tag{5}$$

$$\vartheta = 2\left[\arctan\left(\tan\left(\frac{\vartheta_o}{2} + \frac{\pi}{4}\right)e^{T/n}\right) - \frac{\pi}{4}\right] \tag{6}$$

For high and low gravity, Fig. 4 plots the relative trajectories

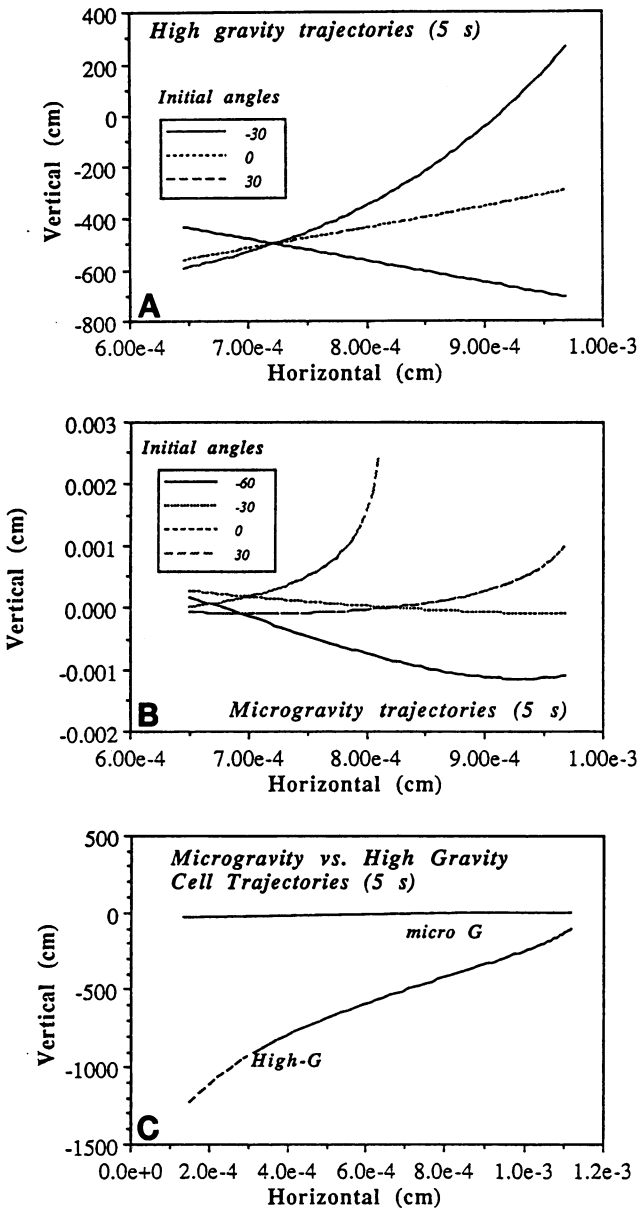

FIGURE 4 Calculated two-dimensional swimming trajectories for various initially specified angles and at two different gravity levels. *A*) unit gravity; *B*) microgravity; *C*) comparison between microgravity and high-gravity trajectories together for the same initial angle.

in two dimensions at different initial angles. High gravity acts to contract the arc of upward swimming and thus more strongly orient vertical cell directions. A summary of the calculated vector trajectories is shown in Fig. 5.

Three-dimensional helical effects

The principal difference related to two- and three-dimensional gravity effects on single-cell swimming trajectories is the gyration angle of the actual (3-D) helicoidal trajectory of the cell. This effect can be captured most transparently in the following mathematical formula for the time evolution of the cell trajectory along the horizontal (x) coordinate

$$x(t) = (a\cos(\omega t) - a)\cos\vartheta + vt\sin\vartheta \qquad (7)$$

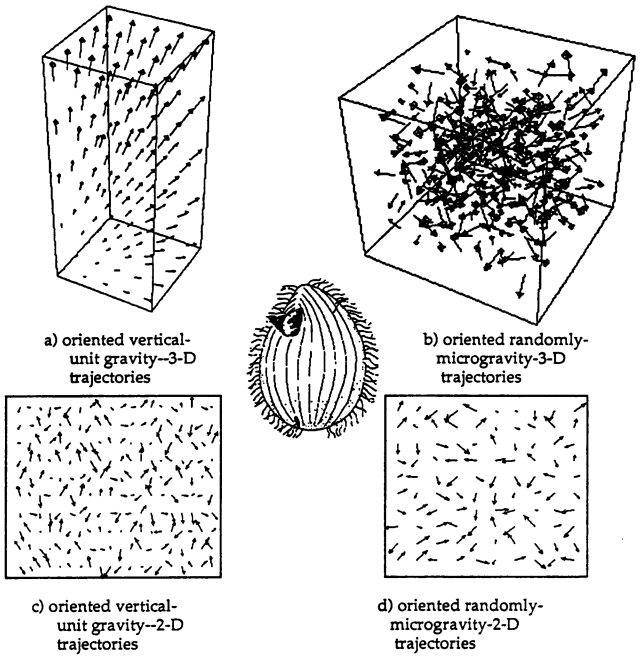
a) oriented vertical-unit gravity--3-D trajectories
b) oriented randomly-microgravity-3-D trajectories
c) oriented vertical-unit gravity--2-D trajectories
d) oriented randomly-microgravity-2-D trajectories

FIGURE 5 A summary of calculated vector trajectories.

for the vertical (y) coordinate

$$y(t) = (-a\cos(\omega t) - a)\sin\vartheta + vt\cos\vartheta - ut \qquad (8)$$

and where v is the swimming velocity (100 μm/s); u is the sedimentation velocity (Stokes, $u = 2(\rho - \rho_0)a^2g/9\eta$); ω is the angular velocity of gyration (0.13 s^{-1}); ϑ is the angle between vertical and instantaneous trajectories. The parametric plot of the cell trajectory (vector $\mathbf{v}=\{x(t), y(t), t\}$) is shown for various conditions in Fig. 6. In this simple model, the effect of gravity becomes most pronounced through the Stokes' sedimentation, where u is linearly proportional to gravity. Gravity has relatively complex effects in three dimensions owing to the change in the gyration properties of a swimming trajectory.

Lift and drag for finding a cell's optimal angle of attack

A final effect worthy of consideration is the effect of aerodynamic forces (Fig. 7) on *Tetrahymena*. As gravity diminishes in significance, aerodynamic effects such as lift and drag become more important for an obliquely oriented swimmer. The fluid contribution to swimming is composed of drag and lift which, in addition to orienting the swimmer, may also contribute to its helical or turning motion directly (Kessler, 1985a,b,1986; Hill et al., 1989; Noever, 1991). For the drag force,

$$F_D = \tfrac{1}{2}\rho A v^2 C_D \qquad (9)$$

where A is the cross-sectional area, C_D is the drag coefficient (assumed constant), v is the swimming velocity and ρ is the relative water density.

Similarly for the lift force,

$$F_L = \tfrac{1}{2}\rho A v^2 C_L \qquad (10)$$

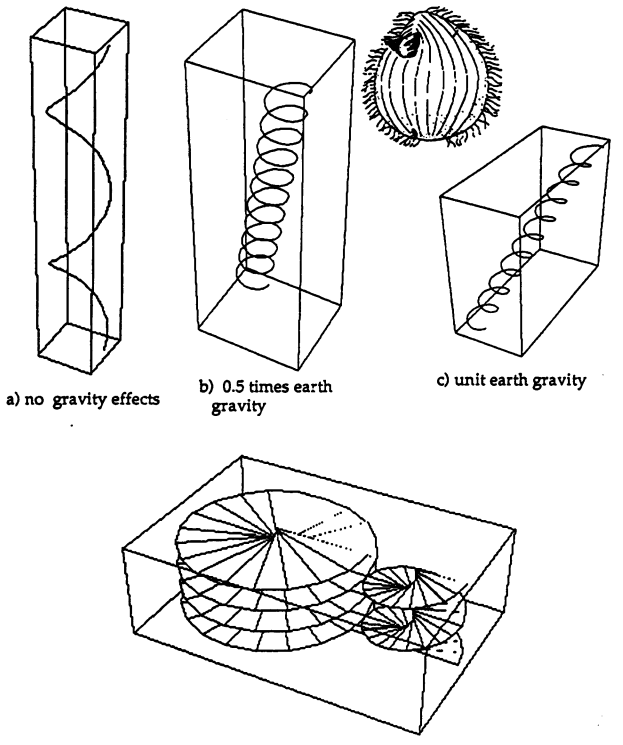
a) no gravity effects
b) 0.5 times earth gravity
c) unit earth gravity
d)Direct comparison of angular orientation as a function of gravity; Left=0.5 times unit gravity; Right=unit earth gravity

FIGURE 6 Calculated three-dimensional trajectories for single swimming cells, which trace a helical swimming trajectory.

where C_L is the lift coefficient. The following calculations were done assuming that the lift coefficient varies inversely with velocity, so the lift force is linear. The ballistic trajectory of an aerodynamic single cell can be described with standard force balances as

$$M\frac{d^2x}{dt^2} = -F_D\cos\vartheta - F_L\sin\vartheta, \quad (11)$$

$$M\frac{d^2y}{dt^2} = -F_D\sin\vartheta - F_L\cos\vartheta - mg. \quad (12)$$

The ratio between aerodynamic and gravity forces can now be simply formulated as

$$\frac{a}{g} = \frac{\text{aerodynamic forces}}{\text{gravity forces}} = \frac{\rho A v^2}{2Mg}(C_D^2 + C_L^2)^{1/2}. \quad (13)$$

The form of Eq. 13 is interesting, since as gravity decreases, the role of drag and lift becomes increasingly important.

The equations of motion can be solved and compared as a function of gravity for three cases: 1) linear drag, 2) quadratic drag, and 3) lift and linear drag. Even in this simplified model of single-cell orientation, only the first case is analytically solvable. The latter two cases will require numerical integration. Eqs. 11 and 12 were solved numerically using a Runge-Kutta method with step size 0.2. For linear approximations, the cell trajectories cannot simulate ciliary beating, complex swimming behavior (sliding, snaking, or writhing motion) or chemoreception. For each case and at different gravity levels, the ballistic horizontal and vertical velocities are shown in Fig. 8.

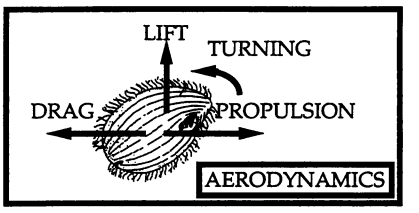

FIGURE 7 Schematic of aerodynamic forces on an obliquely aligned cell.

As shown in Fig. 7, the biological effect of gravity can be seen as a shift in the vertical velocity (via sedimentation) for each case, but most interestingly the effect of aerodynamic lift and drag act together to skew the velocity vectors. It may turn out that the most intriguing aspect of low-gravity work on single cells is not the explanation of gravity reception itself, but the isolation and relative magnification of complex aerodynamic forces. The cell trajectories reflect this aerodynamic orientation effect succinctly. We conclude by noting the significance of aerodynamics when gravity is diminished; the simple model suggests that a repetition of the low-gravity simulation may show preferential cell orientation (either upstream or downstream) as aerodynamic forces operate in a flowing suspension.

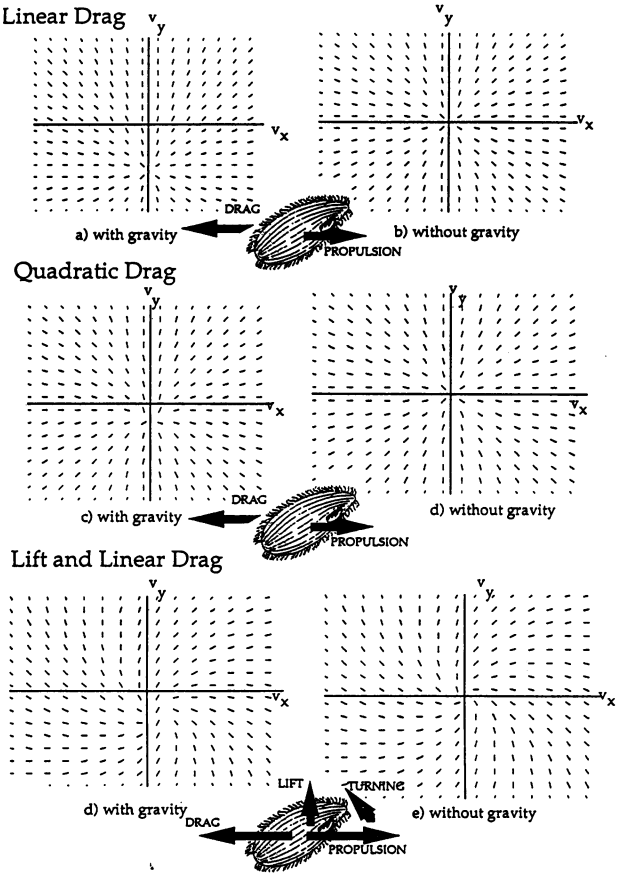

FIGURE 8 Lift and drag forces and the determination of gravity-dependent angle of attack.

DISCUSSION

The experimental measurement of average cell swimming direction in variable gravity has been determined. In airplane experiments, repeated application of variable gravity can isolate biological effects of acceleration and begin to quantify their importance relative to competing influences of aerodynamic forces, light, chemicals, hydrostatic pressure, oxygen, and other physiological gradients. *Tetrahymena* is shown to swim preferentially upward in a gravity field. One appealing aspect of the experiments for gravity sensing is the speed of cell reorientation; organisms are observed to randomize their statistical swimming direction within 20 s in low gravity (0.01 g; g = 980 cm/s).

Unanswered questions for gravity reception

In previous airplane experiments, vertical mixing in dense suspensions of mobile cells was found to depend on the gravity level. These results (Noever, 1991) showed that the absence of gravity precludes vertical mixing of the cell and ruled out competing mechanisms such as oxygen attraction (Shvirst et al., 1984) and cell swarming as required conditions for vertical orientation. Oriented mobility itself was the key component of vertical mixing in dense suspensions.

The biological basis for the action of gravity can be accounted for as motion arising from swimming when coupled to aerodynamic forces in water. Non-motile cells and inanimate objects, although subject to diffusion and viscous drag, do not display any consistent orientation behavior in these experiments compared with motile cells. These results suggest a rather strong interaction between upward swimming and gravity. Gravity itself as an actively varied laboratory parameter cannot elucidate any deeper biological mechanism without, e.g., 1) a genetic variant of *Tetrahymena* that does not show geotaxis, 2) starved cultures which in paramecium have received anecdotal references to a higher center of gravity and thus a reduced tendency to orient bottom down (smaller geotaxic effects), or 3) a detailed biochemical pathway for gravity reception. To the authors' knowledge, none of these experimental descriptions are presently available in the literature and more work in this area would be most welcome.

CONCLUSIONS

To summarize, the current study finds the following. 1) Gravity preferentially directs cell swimming upward (negative geotaxis) in *Tetrahymena* suspensions with a statistically definable preference for gravity orientation centered on 90° (vertical). 2) Vertical migration patterns of organisms in various gravity levels can be characterized by a) their lack of directional correlation (randomization); b) their rapid decay time (<20 s) for upward orientation; and c) their rapid recovery of oriented swimming when normal gravity returns. 3) Vertical orientation does not require oxygen gradients since all experiments were conducted in sealed chambers without bubbles or air gaps.

Vertical migration patterns of single cells are a critical feature of their lifecycle. For plankton, understanding the link between gravity and vertical migration may shed light on interesting models of ocean-atmospheric exchange of gases (Harrison and Caverhill, 1991; Winet and Jahn, 1972) and related issues of ocean ecology (Winet, 1975; Ryther, 1969) and climate modeling (Williamson and Gribbin, 1991).

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